Notes on the Life-History of Trypanosoma gambiense, *etc.*

By Muriel Robertson.

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(Abstract.)

The following is a brief account of some of the more salient features in the life-cycle of *Trypanosoma gambiense*. The results are drawn from a large number of experiments carried out at the Mpumu Laboratory in 1911 and 1912. The present paper is in the nature of a very brief synopsis, and is not a full account of the experiments and conclusions.

I. *Endogenous Cycle in the Blood.*

The part of the life-history of *T. gambiense* spent in the vertebrate—the experiments were carried out with monkeys—is characterised, as is well known, by a marked fluctuation in the numbers of parasites present in the blood. The individuals show a wide range of variation in length and breadth. During the depressed periods, the few parasites present are of the short, relatively broad type. Periods of increase are characterised by the appearance in addition of the intermediate and long slender forms. The latter are the individuals about to divide. The short form may be looked upon as the adult blood-type, and is usually the most numerous form present, except during the periods of rapid multiplication. These periods set in regularly in every revolution of the cycle during the earlier months of the disease; their recurrence is less marked in the later stages.

The short forms appear to be responsible for carrying on the infection in the *Glossina*, and the blood of a monkey is only infective to fly when these forms are present in sufficient numbers and in a suitable physiological condition, *i.e.* not suffering from exhaustion. Intracellular multiplicative phases do not occur in the lung, liver, or spleen of monkeys.

Rounded, non-flagellate individuals are occasionally found in the liver and lung, apparently between the cells, but it is not perfectly clear whether they may not be in rare cases within the cells. They appear at the time when the trypanosomes are being destroyed, before the depressed periods of the endogenous cycle, but have only been found in a teeming infection examined during the earlier months of the disease. These creatures are apparently about to be destroyed, but their survival in very small numbers as latent forms cannot be entirely excluded.

No sexual differentiation of any kind is to be observed among the blood-types. The differentiation into long and short forms is a phenomenon of growth and division, and is not an expression of sex.

A wet fixation of the blood-films in corrosive alcohol, and subsequent staining and mounting without drying in air, gives the nuclear picture shown in figs. 25 and 26. This is in accord with the observations upon live specimens, and is identical with that found in other trypanosomes studied by similar methods. A division-stage is shown in fig. 27.

II. *Exogenous Cycle in the Fly.*

A great deal of information can be obtained from a close study of the conditions in the early days of the cycle as to what are the factors inhibiting the development of the trypanosomes in the *Glossina*. I do not, however, propose to discuss these here; suffice it for my present purpose to take those cases in which trypanosomes have established themselves, and to trace the usual development.

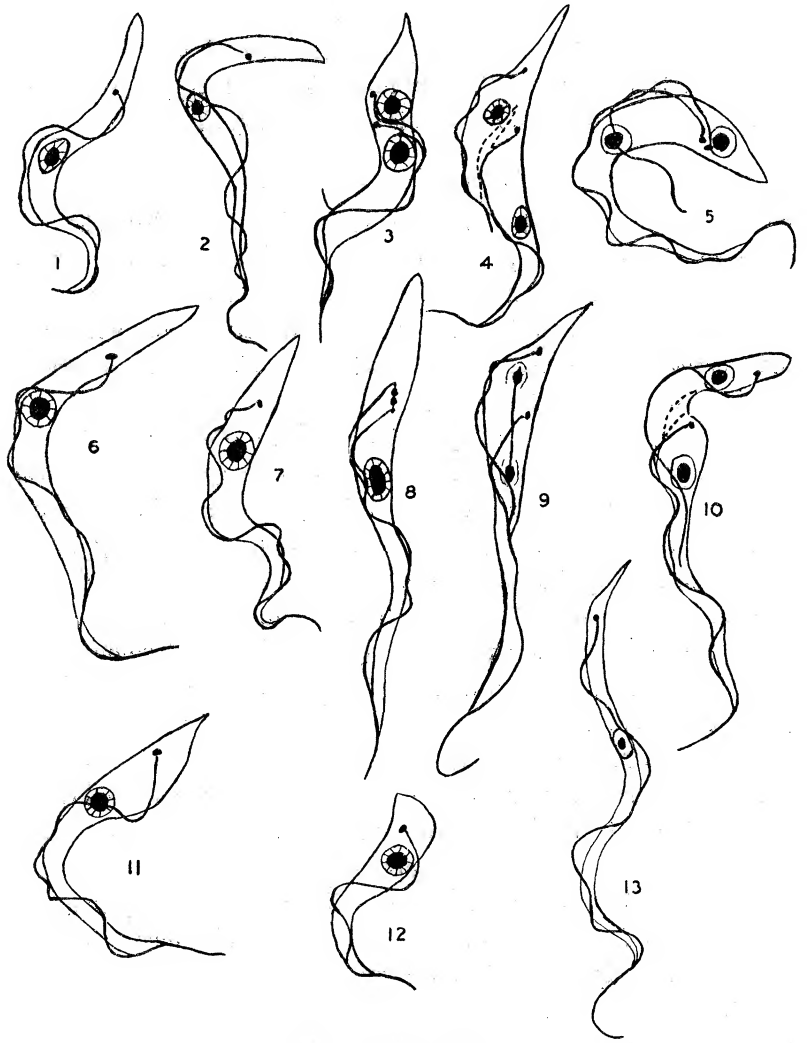
While the series of changes undergone in the *Glossina* up to the time when it becomes infective to clean vertebrates is very definite and constant, nevertheless, the duration in time of this cycle varies in different cases within the limits of more than a fortnight. This must naturally be borne in mind when considering the successive stages in the cycle of any given fly.

The trypanosomes never attach themselves while in the gut, nor do they ever disappear from this situation at any period; the development occurs free in the lumen of the alimentary canal from the very start. At no period do the parasites enter the body-cells of the host, nor do they penetrate through the gut-wall into the body cavity.

The earliest processes that take place in the fly are characterised by a slight and rather indefinite change of form (figs. 1 and 2). Broad, slender and degenerating specimens are all present, but only the broader types are ever found in division at this early stage. These first divisions (figs. 3 and 5) are remarkable in that they show a suppressed crithidial phase in the young individual. This disappears before the separation of the two products (fig. 4). The peculiarity just noticed does not occur in the later divisions, and has never been observed after the 10th day. The gut stages do not show any other crithidial phase. The trypanosomes usually start developing in the middle or posterior intestine (mid-gut), and by the 7th to 10th day there are a large number of trypanosomes present, showing the general features depicted in figs. 6–12. Division goes on rapidly, and the nature of this process is shown in figs. 8–10.

It will be observed than the granule at the base of the flagellum

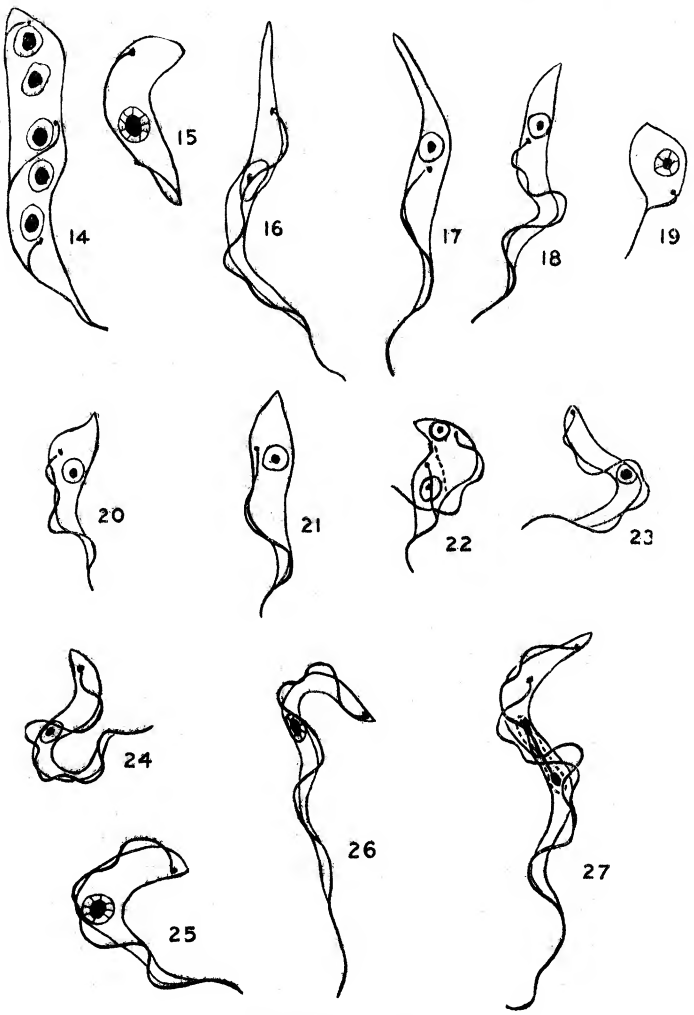
(blepharoplast of Minchin) plays the *rôle* of centrosome in the division of the kinetonucleus. It must also be noted that the division is not really longitudinal but practically transverse, the plane of division being at right angles to the long axis of the parent individual (fig. 10). Division is often unequal.



Magnification 2500.

Multiplication proceeds until the whole of the middle and hinder and part of the anterior intestine is filled with parasites. Very slender long forms are developed about this period (8th to 18th day or thereabouts) and these gradually pass forward into the proventriculus. This slender type (fig. 13) is essentially the proventricular form and is the culmination of the development

in the gut. The trypanosomes may overflow into the sucking-stomach or crop, but are not permanently established there. The flagellates are, moreover, unable to retain their position in the proventriculus if the fly is subjected to a fast of a considerable duration, such as any period exceeding two or three days.



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Multiple Forms.—Up to about the 10th or 15th day of the cycle multiple forms such as those shown in fig. 14 may be seen in certain cases. The evidence is largely in favour of these being degenerative stages but is not sufficient to entirely exclude the possibility that some of them (compare fig. 15) may not be involution-forms or resting phases capable of further development

and activity. Proventricular forms when injected into clean monkeys do not produce infection.

Invasion of the Salivary Glands.—The long slender forms from the proventriculus come forward into the hypopharynx in small numbers at a time and may be found lying free in this situation in carefully dissected specimens before the glands are infected. From the hypopharynx they pass back along the narrow ducts of the salivary glands and it is not at all a rare occurrence to find trypanosomes in the ducts of the glands in 16- to 30-day flies when the rest of the glands show no flagellates at all. The trypanosomes reach the glands as long slender forms and attach themselves where the duct joins on to the slightly broader part which leads to the glandular portion proper. They become much shortened and very much broader and assume the crithidial condition shown in figs. 16–21. They break free occasionally but seem to attach themselves again. Multiplication (fig. 22) occurs and the trypanosomes gradually invade the whole gland; new specimens keep on arriving from the hypopharynx. The short dumpy crithidial forms develop into trypanosomes almost identical with the blood-forms but often a little below the normal adult length (figs. 23 and 24). These trypanosomes are found swimming free in the lumen of the glands and there is the strongest presumptive evidence for considering that these are the types that produce the infection in the vertebrate.

Not only is this second development in the glands necessary to produce an infective fly, but from a number of considerations, amongst others the appearance here of the very clear and definite crithidial stages, it may be held that the development in the glands is the really essential part of the whole cycle. The development in the gut may be considered as a somewhat indifferent multiplication—a mechanical device to enable the trypanosomes to establish themselves in sufficient numbers in contact with the salivary fluid, which alone, in the *Glossina*, seems able to stimulate the trypanosomes to the apparently essential reversion to the crithidial type.

Conjugation.—Sexual differentiation has not been observed at any part of the cycle; this is not, however, a characteristic feature of flagellate life-histories. Isogamy seems to be usual among the group. The direct evidence of conjugation is slight and not sufficiently convincing. General theoretical considerations are, however, very strongly in favour of some such process occurring, and from comparative evidence drawn from the consideration of the cycles of *T. nanum* and *T. vivax* it seems possible that the sexual part of the cycle might take place in the salivary glands.

It is obvious that much of the foregoing work has been simply to carry somewhat further the researches of Minchin, Roubaud, Bruce, and Kleine,

more especially those of the two last-named workers. There are no serious discrepancies between the cycle in the fly sketched by Bruce, Hamerton, and Bateman and that described above, except that I consider the fly history to be in reality a double development. In many points my work is also in agreement with that of Kleine and Taute,* except that I do not consider that the "male" forms described by them play any important part in the cycle. A further discrepancy consists in the view held by the latter authors at the time of writing their paper in regard to the salivary gland phases being a non-essential part of the cycle. My interpretation of the endogenous cycle in the blood of the vertebrate is at present, so far as I am aware, unconfirmed by other workers, largely, I imagine, owing to the fact that the interest has been concentrated for some time past on the appearances in the fly rather than on those in the vertebrate.

On the Comparative Anatomy and Affinities of the Araucarineæ.

By Prof. Robert Boyd Thomson, University of Toronto.

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(Abstract.)

From a study of the anatomy of the different regions of the plant, evidence is found of the relationship of the Araucarineæ to the Cordaitales.

In the first place, the presence of a leaf gap opposite the outgoing foliar trace, in all forms whether the leaf be large or small, is taken as indicating the Pteropsid ancestry of these forms and is considered of sufficient importance to preclude the possibility of the Lycopsid connection of the Araucarineæ, of which view Seward has been the recent exponent. The presence of a gap in the cone and in the seedling seems to put the question beyond doubt, since this indicates the *ancestral presence* of a leaf gap.

One evidence of relationship to the Cordaitales is found in the retention of Cordaitean pitting of the tracheids in the different regions of the plant which are recognised as primitive, in the cone especially, where the pitting may be as much as 5-seriate, the pits, alternate, hexagonal and extending from end to end of the tracheid as in the Pteridosperms and the primitive members of the

* 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' vol. 31, part 2.